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# The effects of gallic/ferulic/caffeic acids on colour intensification and anthocyanin stability

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ABSTRACT

The mechanism by which copigments stabilize colour, by protecting anthocyanin chromophores from nucleophilic attack, seems well accepted. This study was to determine effects of gallic/ferulic/caffeic acids on colour intensification and anthocyanin stability. Molecular dynamics simulations were applied to explore molecular interactions. Phenolic acids intensified the colour by 19% ~ 27%. Colour fading during heating followed first-order reactions with half-lives of 3.66, 9.64, 3.50, and 3.39 h, whereas anthocyanin degradation, determined by the pH differential method (or HPLC-PDA), followed second-order reactions with half-lives of 3.29 (3.40), 3.43 (3.39), 2.29 (0.39), and 2.72 (0.32) h alone or with gallic/ferulic/caffeic acids, respectively, suggesting that anthocyanin degradation was faster than the colour fading. The strongest protection of gallic acids might be attributed to the shortest distance (4.37 Å) of its aromatic ring to the anthocyanin (AC) panel. Hyperchromic effects induced by phenolic acids were pronounced and they obscured the accelerated anthocyanin degradation due to self-association interruption.



## 1. Introduction

The main challenge in the use of anthocyanins as natural food colorants is their relatively low stability. Anthocyanins owe their colour to the high resonance of a fully conjugated 10-electron A-C ring system, with some contribution by the B ring as well. If the resonance structure is disrupted, the colour is lost (Brouillard, 1982). The stability of anthocyanins is determined mainly by their chemical structure and also depends on a combination of environmental factors, including temperature, light, presence of other phenolic compounds, metal ions, ascorbic acid, and oxygen (Delgado-Vargas & Paredes-López, 2003; Shahidi & Naczk, 2004).

Copigmentation represents an important factor in anthocyanin chromophore stabilization (Boulton, 2001; Mazza & Brouillard, 1990; Trouillas et al., 2016). The anthocyanin copigmentation, in model solutions and in wines, has been evaluated and interpreted by the visible $\lambda_{max}$ or differential colorimetry, both in model solutions and in wines (Chung, Rojanasasithara, Mutilangi, & McClements, 2017; García Marino, Escudero-Gilete, Heredia, Escribano-Bailón, & Rivas-Gonzalo, 2013; Gordillo et al., 2015; Navruz, Türkyılmaz, & Özkan, 2016). Anthocyanins are more stable in an aquatic system *via* intermolecular copigmentation with other compounds (Mazza & Brouillard, 1990). The compounds or copigments that can interact with anthocyanins include simple phenols, such as catechin (Dangles & Brouillard, 1992; Mazza & Brouillard,

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1987), flavanol (McDougall, Gordon, Brennan, & Stewart, 2005), chlorogenic acid, caffeic acid, or rutin (Davies & Mazza, 1993) and polyphenols, such as tannins (Remy, Fulcrand, Labarbe, Cheynier, & Moutounet, 2000; Salas, Fulcrand, Meudec, & Cheynier, 2003; Thorngate & Singleton, 1994).

The instant π-π overlap, dipole-dipole interactions, and hydrogen bonding are main molecular interactions of anthocyanin complexation with copigments (Dangles & Brouillard, 1992). The stacking of the copigment molecule on the planar polarizable nuclei of the coloured anthocyanin forms (flavylium ion or quinonoid forms) prevents the nucleophilic attack of water at position 2 of the pyrylium nucleus, leading to colourless hemiketal and chalcone forms (Davies & Mazza, 1993; Mazza & Brouillard, 1990). The anthocyanin self-association also protects their molecular stability (Boulton, 2001). However, it is not clear whether the anthocyanin association with phenolic acids would be better than their self-association to protect pigments from the water nucleophilic attack.

The aims of this study were to investigate effects of gallic/ferulic/caffeic acids on anthocyanins from purple sweet potatoes and their colour, and to explore the mechanism underling the interaction between anthocyanins and phenolic acids. The dynamic changes in colour and anthocyanins were monitored during heating and the intermolecular interactions of peonidin 3-*O*-(2-*O*-β-d-glucopyranosyl-β-d-glucopyranoide)-5-*O*-β-d-glucopyranoside with gallic/ferulic/caffeic acids were explored, using molecular dynamics simulation.

## 2. Materials and methods

### 2.1. Materials and reagents

Purple sweet potatoes (PSP; *Ipomoea batatas*) were purchased at a local market in Shanghai, China. Ferulic, gallic, and caffeic acids were purchased from Shanghai Pureone Biotechnology (Shanghai, China). All other chemicals were purchased from Sigma-Aldrich (Shanghai, China).

### 2.2. Extraction and purification of anthocyanins

The extraction and purification of PSP anthocyanins were performed as described by Jing et al. (2014). Fresh purple sweet potatoes (5 kg) were ground into powder, which was immersed in 10 L of methanol containing 0.01% HCl and extracted for 2 h. The extracts were applied to a 600 cm × 50 cm Amberlite XAD-7HP column (Huideyi, Beijing, China) and washed with 0.01% HCl in water to remove water-soluble compounds. The anthocyanin fraction was eluted with 0.01% HCl in ethanol. The anthocyanin fraction was applied to a 100 cm × 2.5 cm open column packed with Sephadex LH-20 and separated by 50% aqueous ethanol containing 0.01% HCl. The anthocyanin fractions were further purified in an Agilent preparative HPLC system equipped with a semi-preparative ZORBAX Eclipse XDB-$C_{18}$ column. The anthocyanin peak fractions were collected and evaporated to dryness under a nitrogen-blow evaporator. The purity of the anthocyanins obtained from purple sweet potato was >30% (w/w).

### 2.3. Thermal stability of copigmentation

The pigment stability of PSP anthocyanins dissolved in pH 3.2 buffer (0.06 M sodium acetate and 0.02 M phosphoric acid) was evaluated, using an accelerated stability test at 95 °C. The four treated solutions containing 22.5 mg monomeric anthocyanins/l of PSP anthocyanin extract alone or with additional gallic acid (851 mg), ferulic acid (971 mg), or caffeic acid (901 mg) were prepared to satisfy the 1:100 M ratio of cyanidin-3-glucoside and phenolic acid. A series of test tubes was filled with 10 ml of each solution, closed with screw caps and covered with aluminium foil. The tubes were then immersed in a water bath at 95 °C. Twelve tubes (three replicates for each solution) were prepared and sampled at regular time intervals (0, 0.5, 1, 2, 5, 10, and 15 h) and rapidly cooled to room temperature for analysis.

The absorption spectra were recorded, using an L5S UV–visible spectrophotometer (Shanghai Analytical Instrument, China), by scanning the visible range from 400 to 800 nm. A possible hypochromic effect was detected as an increase in the absorbance value at $\lambda_{max}$ and a bathochromic shift as a shift in the wavelength (nm) of $\lambda_{max}$. Samples were stored in a refrigerator and analyzed for monomeric anthocyanins within one day.

### 2.4. Total monomeric anthocyanins

The total monomeric anthocyanins in the PSP extracts was determined by the pH differential method (Giusti & Wrolstad, 2001). An L5S UV–visible spectrophotometer (Shanghai Analytical Instrument, China) was used to read the absorbance at 520 and 700 nm. Total monomeric anthocyanins were expressed as cyanidin-3-glucoside, using a molecular weight of 449.2 and a molar absorptivity of 26,900 $l \times cm^{-1}$ $mg^{-1}$. Cuvettes with a 1 cm path length were used. Measurements were performed in triplicate.

### 2.5. UPLC-ESI-HRMS analysis of PSP anthocyanins

The anthocyanins were separated by an ACQUITY UPLC system (Waters, MA, USA) equipped with an Acquity BEH $C_{18}$ column (1.7 μm, 100 mm × 2.1 mm i.d., Waters, MA, USA). The analysis was conducted at a flow rate of 0.4 ml/min. Mobile phase A was 0.1% formic acid in water (v/v), and mobile phase B was acetonitrile. The following gradient programme was used for the mobile phases: 0–15 min, 5%–20% B; 15–20 min, 20–40% B; 20–22 min, 40–85%. Spectral information was collected over the wavelength range of 200–800 nm.

The LC-HRMS system consisted of a Waters Micromass Q-TOF Premier mass spectrometer equipped with an electrospray interface (Waters Corporation, Milford, MA) at the Instrumental Analysis Center of Shanghai Jiao Tong University. The positive ionization mode was used for the detection of anthocyanins. The applied electrospray/ion optics parameters were set as follows: capillary voltage, 3.0 kV; sampling cone, 35 V; collision energy, 4 eV; source temperature, 115 °C; desolvation temperature, 250 °C; desolvation gas flow, 300 L/h. The scan time was 0.5 s in an *m*/*z* range of 50–1500 au.

### 2.6. Quantitative analysis of individual PSP anthocyanins

The identification of monomeric anthocyanins in PSPs during thermal treatment was performed, using a LC-2030C HPLC system (Shimadzu, Japan) equipped with a binary solvent delivery system, an online vacuum degasser, a diode array detection (PDA) system, an automatic sampler, a thermostatically controlled column compartment and a Shimadzu LabSolutions workstation. Separation was achieved by reverse phase elution on an InertSustain $C_{18}$ column (5 μm, 250 mm × 4.6 mm i.d.). The analysis was conducted at a flow rate of 1 ml/min. The injection volume was 20 μl. The mobile phase consisted of 1% formic acid in water (v/v; eluent A) and of acetonitrile (eluent B). The gradient programme was as follows: 0–5 min, 10% B; 5–20 min, 10–15% B; 20–30 min, 15–20% B; 30–40 min, 20–25%B; 40–45 min, 25–40% B; 45–50 min, 40–60% B. Spectral information was collected over the wavelength range of 200–800 nm, and the detection wavelength was set at 530 nm, whereas the total absorbance of anthocyanin peaks was recorded.